Reed (W.) & Carroll (J.)

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WALTER REED, M.D,

SURGEON, UNITED STATES ARMY

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JAMES CARROLL, M.D.,

ACTING ASSISTANT-SURGEON, UNITED STATES ARMY.

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WERE it not for the fact that the general practitioner of medicine in this country takes such a lively interest in all that pertains to the causation of yellow fever, and might construe our silence as an admission that we had followed faulty methods in our work, we would not at this time reply to the paper of G. Sanarelli, entitled "Some Observations and Controversial Remarks on the Specific Cause of Yellow Fever," which appeared in the MEDICAL NEWS under date of August 12, 1899. To those who are acquainted with the methods followed in the laboratories of the Army Medical Museum, we believe that we could well afford to leave this matter until such time as our completed observations might be given publication.

We pass by, therefore, as unworthy of comment, Sanarelli's insinuation that we could lend ourselves to the support of any personal controversy between himself and Surgeon-General Sternberg; and further, his advice that we should have "looked up for a moment some good treatise on bacteriology before attempting to launch such a paradox" as that bacillus icteroides should be considered to be a variety of the hog-cholera bacillus.

That our paper should partake somewhat of a controversial character, we very much regret, since we would have much preferred to publish our observations in full, so that our fellow-workers might be placed in possession of the complete details of our experimental work and thus be in a position to judge whether our methods had been good or faulty. But for the reason given at the commencement of this paper and because of the remarkable tone of Sanarelli's article, we must take up what we consider to be a somewhat unpleasant task.

Because we have recorded focal necroses in the liver of guinea-pigs and rabbits inoculated with bacillus icteroides (we might have mentioned necroses in the liver of dogs, which Sanarelli has quite overlooked); and since, in Sanarelli's opinion, we have failed to reproduce that degree of "acute steatosis" which he and others have recorded, we are designated as "victims to some deplorable neglect of technical precautions in the laboratory," and the conclusion is drawn "that a gross and deplorable error was made in their laboratory and that somehow the cultures of the two micro-organisms being experimented with got mixed for want of sufficient attention."

We are willing to admit that such a condition of affairs, as is implied in the latter part of this quotation, is possible in any laboratory; but in this connection we cannot refrain from paying our admiration to Sanarelli's mental acumen in arriving at this conclusion, since he must have realized that, if we had *not* "mixed the two micro-organisms," his claim for the discovery of the specific cause of yellow fever can no longer be sustained.

And here let us avow that we hailed with delight Sanarelli's announcement of the discovery of his bacillus icteroides as the specific agent of yellow fever and earnestly hoped that his work would be confirmed by other observers. When later directed by Surgeon-General Sternberg to carefully compare bacillus *x* with bacillus icteroides and report to him the results of our investigations we were only influenced by the desire to ascertain whether these were distinct bacilli, and if so, to see whether we could confirm Sanarelli's observations in the several animals. That we could be influenced in the slightest degree by Dr. Sternberg in arriving at our conclusions is a grave aspersion upon our reputation as scientific workers.

If it can be proved that we have "mixed" our cultures and hence arrived at incorrect conclusions, we will hasten to admit our mistake. Let us see whether we have been careless in our technical precautions, and hence have, by mistake, inoculated our animals with the bacillus of hog-cholera when we had intended to use bacillus icteroides.

In the first place, before proceeding to consider Sanarelli's criticisms of our work, we desire to state that the culture of bacillus icteroides with which we have made the majority of our observations, was obtained by Dr. Sternberg from Pasteur's laboratory. We are informed by Dr. Sternberg that the package containing this culture was opened by Professor Roux in his (Sternberg's) presence and the culture

handed to the latter. It was delivered to one of us by Dr. Sternberg on the 24th day of September, 1897, and bore the label of the Laboratory of Hygiene, University of Montevideo. We may remark that this culture was not in the shape of the ordinary stroke culture, but contained a number of well isolated colonies, such as would be obtained by inoculating an agar slant with a small drop of the blood of an infected animal. Several of the colonies on this agar slant showed the peculiar appearance of growth which Sanarelli considers as characteristic of bacillus icteroides. It was from one of these so-called characteristic colonies that we made our first transfer. Subsequent transplantations have been made by us at intervals of about four weeks, and have always been labeled "Bacillus Icteroides, Original." We have thus transplanted this original culture on agar, without transmission through animals, for a period of nearly two years. Like our other stock cultures, it has been kept in agar tubes provided with rubber caps and always in a dark closet.

In the second place, at the time that we began our investigations (September, 1897), no culture of the hog-cholera bacillus was in our laboratory, nor did we procure a culture of this bacillus from the Bureau of Animal Industry until the following spring (May, 1898) about seven months after we had begun our work.

We may be excused for particularizing somewhat in this connection in order to set ourselves right before those who are interested in this subject.

Let us then state that the hog-cholera bacillus was one with which neither of us was acquainted, except as to some of its most prominent morphological and biological features, such as size, motility, growth in milk, and in the three sugars; but we had not witnessed its pathological effects in the smaller animals. We had more than once commented on the fact that its action on litmus milk, and in the three sugars was similar to that of bacillus icteroides; but as we were not prepared at that time to look upon bacillus choleræ suis as a secondary invader in human beings, we did not follow up the subject further.

During the month of February, 1898, we had ascertained that the blood-serum of a dog partially immunized against bacillus icteroides, although exercising decided agglutinative action upon the latter, was negative as regards the typhoid bacillus and bacillus coli communis, except in low dilutions. At this time our observations were almost completely interrupted by our duties with the class of the Army Medical School. We had scarcely resumed our work when we were honored with a visit from Dr. Theobald Smith, formerly of the Bureau of Animal Industry, and who did all of the bacteriological work recorded in the "Report on Hog Cholera; Its History, Nature, and Treatment," to whose instructive pages Dr. Sanarelli has so courteously invited our attention. We communicated to Dr. Smith the fact that bacillus *x* (Sternberg) when injected into the circulation of dogs would cause the same general symptoms, and the same extensive hemorrhagic lesions in the stomach and intestine as was produced by bacillus icteroides, and hence that these lesions were not confined to the action of the latter bacillus. Dr. Smith then remarked that in reading Sanarelli's various articles, *he was continually reminded of the hog-cholera bacillus.*

When further told by us that bacillus icteroides produced multiple necroses in the liver of guinea-pigs, he remarked that he was of the opinion *that bacillus icteroides would yet be placed in the hog-cholera group.* Fortified by this expression of opinion by one, than whom no one is more conversant with the hog-cholera bacillus or its pathological action, we for the first time proceeded to procure a culture of bacillus choleræ suis, and to test the action of our dog's serum, with the result already given in our preliminary note. As we had *already*, many times, obtained these focal necroses in guinea-pigs with bacillus icteroides, how could we have "mixed the two micro-organisms?" We could scarcely have mixed what we did not have in our possession. If, as Sanarelli has stated, these "focal necroses are entirely specific for hog-cholera," we had already proven by the production of these that bacillus icteroides was a variety of the hog-cholera bacillus. It is very important to note that cultures taken from these animals gave, in about thirty per cent., colonies similar in all respects to those described by Sanarelli as characteristic of bacillus icteroides.

Turning now to Sanarelli's criticisms of our work, these may be stated as follows:

First. The marked difference in growth on potato of his bacillus and bacillus choleræ suis.

Second. That the toxin of his bacillus resists a temperature of 100° C., while that of the hog-cholera bacillus undergoes modification as low as 60° C., and is entirely destroyed at 100° C. Sanarelli here quotes from Selander's observations.

Third. Dissimilarity of growth in gelatin plates of these two bacilli.

Fourth. That while the hog-cholera bacillus produces a large number of foci of coagulation necrosis (the organ is not stated), these do not occur in animals inoculated with bacillus icteroides.

Fifth. That we have failed to produce acute steatosis, or fatty degeneration of the liver in dogs injected with his bacillus.

As regards the difference in growth on potato

Sanarelli says, "it is sufficient to recall that the bacillus of Salmon forms on potato a luxurious brownish-colored culture somewhat like that of Mauve, while my bacillus icteroides develops on potato completely without color and is scarcely visible."

Salmon and Smith,[1] in their report, implied neglect of which on our part to even casually glance at, has occasioned Sanarelli so much apparent regret, state as follows: "growth on boiled potato when at 95° F., appears as a faint straw-colored deposit within twenty-four hours after inoculation. At 20° C. or 25° C., it appears one or two days later. It slowly spreads in all directions as a layer of perceptible thickness. The color changes to a dark brick-red, or *may remain whitish* (italics our own). In general the growth is darker the more rapidly the potato dries up."

Smith and Moore,[2] in describing the growth of several varieties of the hog-cholera bacillus, state "that there may be slight variations in the depth of color of the growth on potato due to variations in the potatoes used. Now and then potatoes are encountered on which no growth appears. The surface assumes a glistening appearance, but multiplication perceptible to the eye does not take place."

Welch,[3] describing the growth of the hog-cholera bacillus, says: "the growth on potato assumes generally a brownish or yellowish tint, *but it may be white* and *sometimes is indistinct* (italics our own), although microscopically the growth is abundant."

Our own observations show that the hog-cholera bacillus, when transplanted on potato grows, as a rule, as a moist straw-colored or brownish layer spreading over the greater part of the surface of the medium; but that exceptionally it presents a moist, almost colorless layer. Cover-slips from this moist, almost colorless layer show that a marked multiplication of the bacilli has taken place.

It will thus be seen that the growth of the hog-cholera bacillus on potato is variable, and that it does not always produce "a luxurious brownish colored growth," as claimed by Sanarelli.

Our experience with the growth of bacillus icteroides on potato is at variance with that given by Sanarelli. Both of us promptly transferred to various media the original bacillus icteroides turned over to us by Dr. Sternberg on September 24, 1897. As regards the growth on potato, we quote from our notes made on September 29th, 1897, as follows: Dr. Carroll, "a moist brownish growth." Dr. Reed, "grows less rapidly than bacillus *x*, giving a moist, thin, brownish layer, which spreads over the surface of the medium." These were duplicate observations made independently of each other.

Our subsequent observations have shown that bacillus icteroides on potato may grow as a colorless moist layer, or as a faint yellowish layer, or that it may show a decided brownish color.

A. Agramonte,[1] who spent some months in this laboratory comparing a bacillus which he had obtained from yellow-fever cadavers at Santiago, Cuba, during the summer of 1898, with bacillus icteroides, could find no difference between these. He says as regards the growth of this bacillus: "Potato cultures for twenty-four hours at 39° C. gave an abundant pale-cream, almost colorless growth; when compared side by side with others made of bacillus icteroides it was impossible to differentiate them. At the end of a week the cultures remained of the same color; while some of bacillus icteroides had taken on a *brownish* tinge (italics our own), others remained the same.

All of which goes to show that bacillus icteroides does not always grow on potato as a colorless, scarcely visible layer, as claimed by Sanarelli, and hence that he cannot safely base a differentiation on this account between his bacillus and bacillus choleræ suis. It would be well if Sanarelli would recall our experience with the typhoid bacillus. As with that bacillus, so with bacillus icteroides, it may or may not grow on potato as a colorless layer.

With reference to the claim of Sanarelli that the toxin of his bacillus will resist a temperature of 100° C., while that of the bacillus of hog-cholera undergoes modification as low as 60° C., and is entirely destroyed at 100° C., we have no observations of our own to record. We propose to show, however, that his statement concerning the supposed effect of certain temperatures on the toxin of the bacillus choleræ suis, which he quotes from Selander, is entitled to no credence whatever. Indeed, we consider Sanarelli's reference to the work of Selander and Metchnikoff[2] as particularly unfortunate, since subsequent observations have conclusively demonstrated that at that time, 1890–92, neither Selander nor Metchnikoff were working with the hog-cholera bacillus!

We almost feel like suggesting to our distinguished critic that he should follow the advice which he seems so willing to give to others, and spend a brief time in consulting the literature on this subject.

Referring to Selander's investigations, Welch and Clement[3] say: "We wish, however, in this connec-

[1] "Hog Cholera," Bureau of Animal Industry, Washington, 1889.
[2] "Infectious Swine Diseases," Washington, 1894.
[3] *Johns Hopkins Bulletin*, vol. i, No. 1, 1889.

[1] The bacillus icteroides (Sanarelli) and bacillus *x* (Sternberg), *Centralblatt für Bacteriologie*, etc., Band xxv, No. 18–19, 1899.
[2] *Annals de l'Institut Pasteur*, 1890–92.
[3] "Hog Cholera and Swine Plague," Proceedings First Veterinary Congress of America, Chicago, 1893.

tion, to express our conviction that the organism described by Selander in 1890, and probably also that by Metchnikoff later, as the hog-cholera (or swine-pest) bacillus, is not identical with the genuine hog-cholera bacillus as we know it in this country." After recording their inability to confirm the experiments of Selander in increasing the virulence of the supposed hog-cholera bacillus, they say: "Whatever this organism of Selander may have been, we feel convinced that it was not our hog-cholera bacillus, and we base this conclusion upon the results of his experiments in Pasteur's Institute, as published, and upon our experience with the cultures with which he kindly supplied us. . . . We believe Metchnikoff's hog-cholera microbe to be one of the hemorrhagic septicemia bacilli, possibly the swine-plague or schweine-seuche bacillus."

This opinion expressed by Welch and Clement was subsequently proven to be correct by Smith and Moore,[1] to whom Metchnikoff, having confirmed the observations of Selander in increasing the virulence of the supposed hog-cholera bacillus sent the blood of a guinea-pig inoculated with the bacteria in a sealed glass tube. These writers state that "cultivated on various substrata they presented all the characters of swine-plague bacteria as found in this country." Certainly we have not claimed any affinity between the swine-plague bacillus and bacillus icteroides.

Not only does Sanarelli, in quoting from the work of Selander concerning the effect of varying temperatures upon the toxin of Selander's bacillus, confound the bacillus of swine-plague with the bacillus of hog-cholera, but he also displays the most astonishing want of acquaintance with the biological characters of the last-named bacillus. He says: "We may recall, besides, that colonies of the two forms of bacilli (bacillus icteroides and bacillus choleræ suis) on gelatin plates are as readily differentiable, even at first sight, as it is possible for bacilli to be."

On the contrary, we wish to state that plated, side by side, in gelatin, we have been unable to detect any differences in the colonies of these two bacilli with the naked eye, and that it is only by means of the microscope that faint differences can, as a rule, be made out, such as a slightly increased radiation in the colonies of bacilli icteroides, as compared with those of bacillus choleræ suis. We are, therefore, constrained to the opinion that Dr. Sanarelli has never plated on gelatin the genuine hog-cholera bacillus, such as we know it in this country.

Referring to the focal necroses found by ourselves in the liver of guinea-pigs and rabbits inoculated with bacillus icteroides, Sanarelli says: "I am very much surprised that Reed and Carroll should have found this lesion, and should have betrayed seemingly good faith in describing it so carefully, without realizing that the lesion is entirely specific to hog-cholera and limited to that affection, and is completely unknown in infections with bacillus icteroides."

We can pass by the discourteous part of the foregoing sentence; but we must confess that even had we not succeeded many times in producing these focal necroses in the liver of pigs and rabbits with bacillus icteroides, we were not prepared for the statement that this lesion was "entirely specific to hog-cholera and limited to that affection;" especially since Blachstein[1] had succeeded in producing this lesion in the liver of rabbits by the intravenous injection of bacillus coli communis and bacillus typhi abdominalis; and since, moreover, one of us (Reed[2]) working in Welch's laboratory, had caused the same lesion by injecting cultures of the typhoid bacillus into the mesenteric vein of rabbits.

Blachstein, speaking of the lesions due to bacillus coli communis, says: "The more striking and constant lesions, those most characteristic of the affection are in the bile and liver." Further he says: "only one or two such areas may be noticed, or they may occur in large numbers. . . . These areas occur both on the surface and in the depth of the liver. . . . On microscopical section these areas are found to be places where the liver cells have undergone necrosis." He adds: "These necrotic and inflammatory changes appear to be of essentially the same nature as have been observed after ligation of the common bile duct (Foá, Salvioli, Pick, and others), and occur in other infectious processes, such as after inoculation with typhoid bacilli, and with hog-cholera and schweine-pest bacilli."

Indeed, scattered areas of coagulation necrosis in the livers of these animals have been produced by other bacteria, and notably by such toxalbumens as abrin and ricin, in the hands of Flexner,[3] and we know, as stated by the latter, the close relationship existing between these bodies and certain bacterial toxins.

Again we ask, can Dr. Sanarelli be ignorant of the rich literature relating to the production of multiple focal necroses in the liver of guinea-pigs, rabbits, kittens, etc., by the bacillus diphtheriæ and its toxins? Indeed, in limiting focal necroses in the livers of guinea-pigs and rabbits to the agency of

[1] "Infectious Swine Diseases," Washington, 1894.

[1] "Intravenous Inoculation of Rabbits with the Bacillus Coli Communis and the Bacillus Typhi Abdominalis," *Johns Hopkins Bulletin*, vol. ii, No. 14, 1891.

[2] *Johns Hopkins Hospital Reports*, vol. v, 1895.

[3] "The Pathology of Tox-albumen Intoxication," *Johns Hopkins Hospital Reports*, vol. vi, 1897.

one particular bacterium, Sanarelli displays profound ignorance of the pathology of coagulation necrosis.

Turning to our statement that these lesions are caused by inoculation with bacillus icteroides, we invite attention to the fact that Agramonte,[1] to whom we communicated our observations, obtained the same necroses in pigs injected with a bacillus obtained from yellow-fever cadavers (33 per cent.) at Santiago, and which he identified as bacillus icteroides (Sanarelli). We note also that De Lacerda and Ramos[2] record the finding of "scattered yellow points" (*avec des points jaunes disséminès*) in the liver of one rabbit out of four inoculated with bacillus icteroides and which died on the second day. They say nothing about the microscopic examination of these scattered yellow points.

We have failed to find, as a rule, necroses in the livers of animals dying earlier than the fourth day, although we have generally found them between the fourth and ninth day after inoculation, especially prominent in the livers of guinea-pigs. Our earliest recorded observation was made on November 13, 1897, upon autopsying guinea-pig No. 456, dead on the fourth day after receiving 1 c.c. of a 20-hour bouillon-culture of bacillus icteroides. Concerning the liver, we note: "Organ pale; some recent adhesions, numerous small punctiform areas, yellowish white in color, to be seen under capsule."

With regard to the several observers cited by Sanarelli as in opposition to our findings, we have been unable to obtain the articles written by some of these and hence cannot state just what lesions they did find in the livers of the inoculated animals. Of those accessible to us, we find that Domennica della Rovera[3] states the following as the result of his infection of guinea-pigs with bacillus icteroides: "My experiments have demonstrated as a new finding in the liver, foci of small cells (focolai parvi cellulari) situated in the midst of the lobules; the protoplasm of the liver-cells appear very granular, the nuclei discolored and feebly stained, as well as the nuclei of the cells lining the biliary canals and capillaries." This description of Rovera agrees very well with what we have found microscopically, except that we also find larger foci, whose capillaries are crowded with polymorphonuclear leucocytes, and whose cells stain brightly with eosin, especially toward the more central parts of the area. Sometimes we find an entire lobule the seat of coagulation necrosis. This quotation further shows the accuracy with which Della Rovera has made his observations, and completely refutes the position taken by Sanarelli, *viz.:* that focal necroses are never found in the livers of animals inoculated with bacillus icteroides. Rovera[1] also says concerning lesions found in the livers of rabbits: "I also saw foci of small cells, situated within the lobules, such as I have already recorded for guinea-pigs."

In view of the foregoing observations comfirming our findings as regards the agency of bacillus icteroides in producing focal necroses in the liver of of guinea-pigs and rabbits, we are constained to say that Dr. Sanarelli in emphasizing the specific character of these necroses for the hog-cholera bacillus, again shows a surprisingly limited knowledge of the work which has been recorded in this line. We are quite willing to admit, however, that these necroses are probably more abundantly produced by the hog-cholera bacillus and its varieties, such as bacillus icteroides, than by other bacteria.

This brings us to Sanarelli's fifth criticism of our results, *viz.:* that we "did not succeed in reproducing the acute steatosis, fatty degeneration of the liver in dogs that was always so prominent a feature in my own observations as well as those of Foá" and several other investigators whose names we here omit.

We have endeavored to extract from the literature, as far as the Library of the Surgeon-General's Office would permit, the experiments made upon dogs by various observers, but have not been able to find definite data except as regards the experiments of Sanarelli,[2] De Lacerda and Ramos,[3] to which we add our own results.

We submit these in the following table:

It will be seen that there are eight (8) observations by Sanarelli; twelve (12) by De Lacerda and Ramos, and seven (7) by Reed and Carroll, making a total of twenty-seven (27). Of these, six (6) dogs recovered, leaving twenty-one (21) that died as the result of the intravenous inoculation of the cultures or toxin of bacillus icteroides, or both of these. In one instance, the toxin was injected both intravenously and into the substance of the liver. Omitting one dog, which was not autopsied, we have twenty (20) fatal results, with fatty changes in liver recorded in thirteen (13), or sixty-five per cent. Of these, one, reported by Sanarelli (No. 3), died within three hours and forty minutes after receipt of the injection, and "showed that the hepatic cells were already rich in fat drops." He does not state that there was any fatty degeneration present, and we can well believe that such was not the case.

[1] *Centralblatt für Bacteriologie*, etc., Band xxv, No. 18–19, 1899.
[2] "Le bacille Icteroide et Sa Toxine," *Archives de Medicine Experimentale*, No. 3, 1899.
[3] "Sul bacillo Icteroide" (Sanarelli), *La Reforma Medica*, vol. iii, No. 9, July, 1898.

[1] *Archives de Medicine Experimentale*, No. 3, 1899.
[2] *Il Policlinico*, vol. iv, No. 16 and 18, 1897.
[3] *Archives de Med. Exper.*, vol. ii, No. 3, May, 1899.

INJECTION OF DOGS WITH BACILLUS ICTEROIDES.

Observer.	No.	Quantity received.	Result.	Remarks.
Sanarelli....	1	10 c. c. of a 24-hour bouillon culture, intravenously.	Recovered.	
"	2	Agar culture diluted with 1 c.c. of a bouillon culture, intravenously.	Died during the same night.	Liver congested, but no fatty degeneration.
"	3	Agar culture diluted with 1 c.c. of a bouillon culture, intravenously.	Died in 3 hours and 40 minutes.	Liver shows numerous yellowish spots. Fresh sections in osmic show the hepatic cells already rich in fat drops.
"	4	5 c.c. of a bouillon culture, intravenously.	Died on 16th day.	Intense fatty degeneration of liver.
"	5	Agar culture diluted with bouillon—quantity not stated, intravenously.	Died during the same night.	Liver pale with zones of fatty degeneration.
"	6	2 c.c. of a bouillon culture, intravenously.	Died on 19th day.	Marked fatty degeneration of liver.
"	7	2 c.c. of a bouillon culture, intravenously.	Died on 8th day.	Fatty degeneration of liver.
"	8	5 c.c. of a bouillon culture, intravenously.	Died on 10th day.	Marked fatty degeneration of liver.
De Lacerda and Ramos.	1	1 c.c. of an agar culture diluted with 10 c.c. of sterilized water, intravenously.	Died during the same day.	No change in liver.
" " "	2	10 c.c. of an agar culture diluted with sterilized water, intravenously.	Recovered.	
" " "	3	20 c.c. of a bouillon culture into liver.	Recovered.	
" " "	4	20 c.c. of a bouillon culture 2 days old, intravenously.	Died on 19th day.	No autopsy.
" " "	5	35 c.c. of a bouillon culture 3 days old, intravenously.	Recovered.	
" " "	6	35 c.c. of a bouillon culture 1 day old, intravenously.	Died in 16 hours.	No trace of fatty degeneration.
" " "	7	18 c.c. of a bouillon culture 2 days old, and 38 days later 60 c.c. of toxin, both intravenously.	Died in about 8 hours after the 2nd injection.	Fatty degeneration of liver.
" " "	8	20 c.c. of a bouillon culture and 10 c.c. of the toxin, intravenously.	Died in 24 hours.	Liver and kidney of a blackish color. Nothing said of fatty generation.
" " "	9	60 c.c. of the toxin, intravenously.	Died during the same night.	Intense congestion of all organs. No fatty degeneration.
" " "	10	20 c.c. of the toxin, intravenously, and 20 c.c. into the liver.	Died in 24 hours.	Fatty degeneration of liver, kidneys, and heart.
" " "	11	70 c.c. of the toxin, intravenously.	Died at 1 o'clock of the same night.	Fatty degeneration of liver.
" " "	12	50 c.c. of the toxin, intravenously.	Died on 7th day.	Disseminated points of fatty degeneration but little advanced.
Reed and Carroll.....	1	5 c.c. of a 24-hour bouillon culture, intravenously, Sept. 24, 1897.	Died on 9th day.	Necroses in liver. Moderate fatty degeneration.
" " "	2	5 c.c. of a 24-hour bouillon culture, Nov. 16, 1897, and a second injection of 5 c.c. 14 days later, both intravenously.	Died in 28 minutes after second injection.	Slight fatty degeneration of liver.
" " "	3	2½ c.c. of a 24-hour bouillon culture, intravenously, Dec. 13, 1897.	Died on 9th day.	Marked fatty degeneration of liver.
" " "	4	5 c.c. of a 24-hour bouillon culture, intravenously, Feb. 9, 1898.	Recovered.	
" " "	5	5. c.c. of a 24-hour bouillon culture, intravenously, March 17, 1898.	Died in 52 hours.	Necroses in liver. No fatty degeneration.
" " "	6	5 c.c. of a 24-hour bouillon culture, intravenously, July 1, 1898.	Died in 16 hours.	Necroses in liver. No fatty degeneration.
" " "	7	5 c.c. of a 24-hour bouillon culture, intravenously, July 1, 1898.	Recovered.	

What he found in this liver may readily be found in the normal hepatic cells of dogs in a good condition of health and nutrition. We would, therefore, exclude this case as well as the case (No. 12) recorded by De Lacerda and Ramos, in which they found "disseminated points of fatty degeneration, but little advanced," in the liver. No results of the microscopic examination is given in this case to show what the true character of the "disseminated points" was, whether fatty or necrotic, and since it is not characteristic of fatty degeneration of the liver that it should be found in "disseminated points," we feel justified in also omitting this case. We will not do so, however, in order that we may not be accused of unfairness.

We thus find twelve (12) positive results against eight (8) negative results in dogs injected with bacillus icteroides. Of the positive results five (5) are recorded by Sanarelli in seven (7) animals that died (71.3 per cent.); four (4) by De Lacerda and Ramos in eight (8) dogs autopsied (50 per cent.); and three (3) by Reed and Carroll in five (5) deaths (60 per cent.). It will be seen that the percentage of cases showing fatty degeneration is highest in Sanarelli's experiments, due probable to the fact that his dogs lived longer, but that the percentage reported by De Lacerda and Ramos is less than that reported by ourselves. Since De Lacerda and Ramos are authorities quoted as sustaining Sanarelli in his observations concerning the frequent occurrence of fatty degeneration in dogs injected with bacillus icteroides, and since we have obtained this change in even a larger percentage of cases, it naturally follows that Sanarelli, in criticising us for our failure to produce fatty degeneration in the liver of dogs, was speaking as usual without any knowledge of the true facts, just as we have already pointed out that he has done in the matter of Selander's observations, and in the absurd statement that necroses in the liver of guinea-pigs and rabbits "are entirely specific for hog-cholera!" True we did state, and we wish here to repeat, that, with one exception hereafter to be given, the fatty change found in the liver of these dogs is not comparable to that found in the liver of human beings who have died of yellow fever, and we may add that there is an almost entire absence of that necroses of individual liver cells which is so prominent a feature in the human liver.

Sanarelli speaks as if yellow fever were the only condition in which fat is found in the liver cells. It is hardly necessary to remark that it may be present in considerable quantity in the livers of comparatively healthy individuals. Steatosis may mean little or much; it does not necessarily imply degeneration. Pathologists well know that in yellow fever the change that takes place in the liver is essentially one of *degeneration and necrosis*, in which the hepatic cells appear as denucleated bodies distended with minute fat-droplets and containing only a small amount of protoplasmic débris, or as somewhat refractive bodies, which stain brightly with eosin. Their outlines are very much distorted and the normal beam-work arrangement is no longer seen. Sanarelli's illustrations in Plate 10, Fig. 5, *Il Policlinico*, Nos. 16 and 17, 1897, prove that this degenerative and necrotic condition was not present in the livers of human beings inoculated with the toxin of his bacillus. In his section of dog's liver the arrangement of the liver cells is perfectly preserved, their nuclei stain well, and there is only a moderate deposition of fat in the cell protoplasm.

In connection with the production of fatty degeneration of the liver of dogs injected with bacillus icteroides, we desire to invite attention to the comparative degree of this change found in the case of dogs 531 and 928 of our series of experiments.

Dog No. 531, weight 17 pounds, was originally inoculated on February 9, 1898 with 5 c. cm. of a bouillon-culture of bacillus icteroides, from which he recovered. He was then subjected to intravenous injections of increasing doses until he was able to bear injections of 150 c. cm., of a bouillon-culture of this bacillus. On July 20, 1898, the animal had received 150 c.cm. of the culture, and died a few hours after the injection of the same quantity on July 29th. Thus this dog had received during a period of five (5) months and twenty (20) days, 1042½ c. cm. of a bouillon-culture of bacillus icteroides; yet at autopsy the liver was found to contain only a moderate amount of fat, and upon microscopic examination but slight fatty degeneration of the hepatic cells was to be seen.

On the contrary, dog 928, weight 19 pounds, was injected with cultures of the bacillus choleræ suis for the purpose of immunization, and received a total of 170 c.cm. during a period of four (4) months and five (5) days. The largest dose was 50 c.cm. injected on July 19, 1899, from which the animal died thirty-six hours later. At autopsy the liver was seen to be quite light in color, and upon microscopic examination the hepatic cells were found to be not only the seat of advanced fatty degeneration, but there were, also, to be seen many necrotic liver cells. Sections of this liver, indeed, show a stage of fatty degeneration and necrosis closely approaching that of yellow fever in human beings, and yet this animal was injected with bacillus choleræ suis! Or did we here again commit the deplorable error of "mixing the two micro-organisms"? Rather would we say that the important determining factor

in producing this fatty change was the difference in resistance of these dogs, and was not dependent on the particular variety of the hog-cholera bacillus with which we had injected them.

We observe, with some surprise, that Sanarelli does not comment on the remarkable agglutinative reaction which icteroides serum (supplied to us from Brazil, and presumably from animals inoculated with pure cultures of bacillus icteroides), exerts toward the hog-cholera bacillus. The scientific fact alone, *viz.*, that this serum diluted 100,000 times should arrest the motility and agglutinate another bacillus, should, we think, have momentarily attracted his attention; or is it possible that we here again got our cultures "mixed" and were really subjecting bacillus icteroides to the action of its own serum? Nor does he appear to have been impressed with the fact that the hog-cholera bacillus should so completely reproduce in dogs, not only the clinical picture of vomiting, rectal tenesmus, and profound prostration, but also death with extensive hemorrhagic changes in the stomach and intestines. We trust, however, that he will later procure a culture of the bacillus choleræ suis and give us the result of his observations with it on various animals. In the meanwhile, we beg to observe that Agramonte,[1] working in this laboratory for a period of four months, was unable to detect any differences between the "mixed" culture with which we, unwittingly, supplied him, and a bacillus which he had recovered in Cuba from yellow-fever cadavers, and which he identified as bacillus icteroides (Sanarelli).

Concerning the statement made by us in our preliminary note that bacillus icteroides and bacillus choleræ suis both resisted extremely low temperatures, we had not intended to "set this down as one of the characteristics of bacteria by which they might be recognized," since we did not know any bacterium that would not withstand low temperatures. We were only dealing with *bacillus icteroides as the supposed specific cause of yellow fever*, and since by a natural law the specific agent of this disease is arrested and destroyed by a temperature less protracted, and considerably less in degree, than the temperature required to destroy bacillus icteroides, we were justified in emphasizing this fact. We leave, however, to F. G. Novy, the easy task of answering this part of Sanarelli's article.

At the time we began our experiments with feeding bacillus icteroides to young hogs we were well aware that every precaution should be taken to avoid error in experiments on these animals, as emphasized by Welch and Clement.[2]

[1] *Centralblatt für Bacteriologie*, etc., Band xxv, No. 18–19, 1899.
[2] Proceedings First Veterinary Congress of America, Chicago, 1893.

Our first experiment was made in a room where a dog had already died some weeks before from the intravenous injection of bacillus choleræ suis. On this account we did not attach too much importance to this observation. We proceeded at once, however, to procure a second-story room with cemented floor which had never been used for animal experimentation. We had it thoroughly wiped out with a disinfectant solution; we also covered the windows with mosquito-netting in order to prevent the ingress of flies. A new broom and mop were provided for this room. We placed therein four new wooden boxes, and in each of these we put a young hog on April 6, 1899. These animals had been purchased in open market, and were fat and healthy in appearance. April 8th, after withholding food for twenty-four hours, two of these pigs were fed with a twenty-four-hour-bouillon culture of "bacillus icteroides, original," passed through one guinea-pig for the purpose of ascertaining whether the bacillus grown on agar for a period of seventeen months was still virulent or not. On April 10th, 1899, the remaining two pigs were fed, under like circumstances and with a culture of bacillus icteroides having the same source. The culture of bacillus icteroides was in each case mixed with a small quantity of milk brought directly from the dealer. The temperature of the hogs was taken both before and after feeding, and we were careful to have the thermometer placed in a disinfectant solution immediately after being used. The hands of the attendant were also carefully disinfected on each occasion. Under these conditions, and with these precautions, the four animals promptly sickened and died, as shown in the following table:

Careful cultures were taken in each case from the several organs, the blood, bile, and mesenteric glands. From the liver in one case, and from the mesenteric glands in all other cases, colonies of a small, quite motile bacillus were obtained. This bacillus was promptly agglutinated by high dilutions of Sanarelli's serum. From the other sources cultures were negative.

Now we think that looking at these experiments critically, there were two chances for error to have crept in; but not through lack of technical precautions or neglect on our part. In the first place, having injected the guinea-pig with "bacillus icteroides, original," we might have recovered the hog-cholera bacillus from his blood and organs, but this hardly seems probable. Our cultures were pure from this guinea-pig, and we have every reason to believe that we recovered bacillus icteroides. In the second place, as we only kept our young hogs for a few days before feeding them the culture, we

YOUNG HOGS FED WITH BACILLUS ICTEROIDES.

No.	Date.	Quantity.	Result.	Lesions.	Remarks.
976	Apr. 8, '99.	25 c.c. 24-hour culture.	Died on 9th day.	Diphtheritis of stomach and large intestine.	Diarrhea and fever April 10th, with thin, pea-soup stools. Weakness of hind extremities.
977	Apr. 8, '99.	15 c.c. 24-hour culture.	Died on 6th day.	Ulcers on lips; hyperemia of fundus of stomach; ulcerated Peyers patches; follicular ulcers in large intestine with cork-lining exudate.	Diarrhea and fever, beginning April 10th. Emaciation; loss of strength in hind legs.
978	Apr. 10, '99	20 c.c. 24-hour culture.	Died in 7 days.	Congestion and ulceration of mucosa of stomach. Ulceration and diphtheritis of cecum and colon.	Diarrhea April 11th and succeeding days. Emaciation. Loss of strength in hind extremities.
979	Apr. 10, '99.	15 c.c. 24-hour culture.	Died in 12 days.	Erosions on tongue. Diphtheritis of stomach and esophagus. Ulcers in stomach, small intestine, and cecum. Numerous small circumscribed thickenings in submucous layer of large intestine. The centers of some of these already undergoing necrosis.	Same clinical symptoms, beginning on April 12th.

cannot say that some one of these hogs may not already have been infected with the hog-cholera bacillus. They were bought in an open market, and we kept no controls for these first experiments, not being able to obtain pigs from the same litter. But admitting this chance for error, we can hardly believe that *all* of these hogs were already so badly infected with the hog-cholera bacillus that they should die within twelve days from the feeding, and that the symptoms of illness, such as diarrhea and loss of appetite, should have so promptly appeared after the inoculation with bacillus icteroides. If the hog-cholera bacillus were present in one, it was present in all, and it is remarkable that the disease should have run such an acute course after natural infection. We are informed by the dealer from whom these pigs were purchased that they had been in his possession for about a week when sold to us. He also has stated to us that during the present year, although retaining his hogs for periods varying from a few days to several weeks, he has not had any pig sicken or die on his hands.

We think, therefore, that we were quite justified, as the result of these experiments, in expressing the opinion that bacillus icteroides was a variety of the hog-cholera bacillus. We may state that nothing has occurred since the publication of our preliminary note to cause us to change this opinion; but that our subsequent results, such as the immunization of guinea-pigs with gradually increasing doses of the dead culture of bacillus icteroides against a fatal dose of the hog-cholera bacillus, and securing the same result by injections of the dead cultures of the latter bacillus against a fatal dose of bacillus icteroides, have rather confirmed us in that opinion.

We have also been permitted to study sections of the liver from the case of a soldier who died in Havana during the present year, and from the microscopical findings we are strengthened in the belief that bacillus icteroides should be considered as a secondary invader. From the blood of this case during life, bacillus icteroides was recovered both by the members of the Havana Commission and by A. Agramonte.[1] This bacillus was also obtained on cultures after death by the same observers. It is important to note, however, that from the spleen, after death, the typhoid bacillus was also obtained by Agramonte. Careful study of sections of the liver in this case shows an entire absence of fatty degeneration, while many areas of necrosis, such as characterize the liver of typhoid fever, are to be plainly seen. In other words, bacillus icteroides, though present during life in this patient's circulation, failed to bring about that "acute steatosis" upon which Sanarelli lays so much stress.

We hope, at an early date, to present our complete report to Surgeon-General Sternberg.

[1] *Centralblatt für Bakteriologie*, etc., Band xxv, No. 18–19, 1899.